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## FERTILE STROMATA OF *CAMAROPS PETERSII* IN CULTURE

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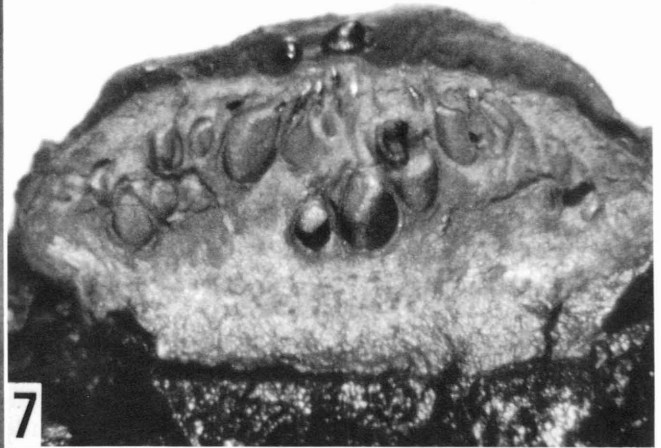
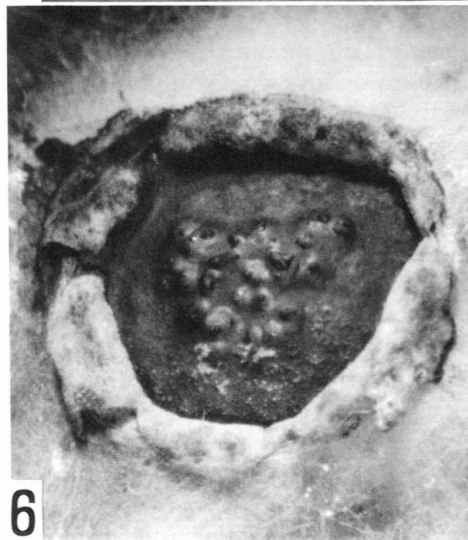
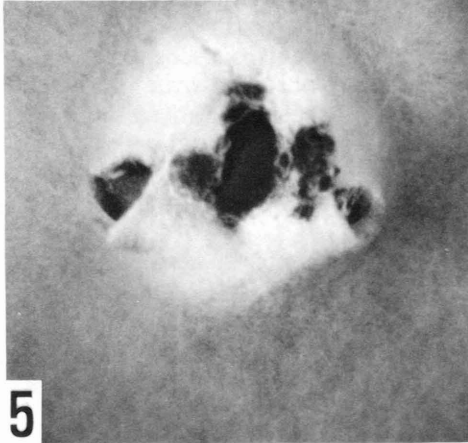
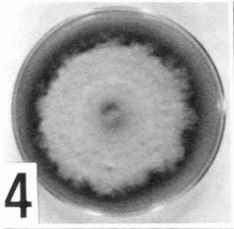
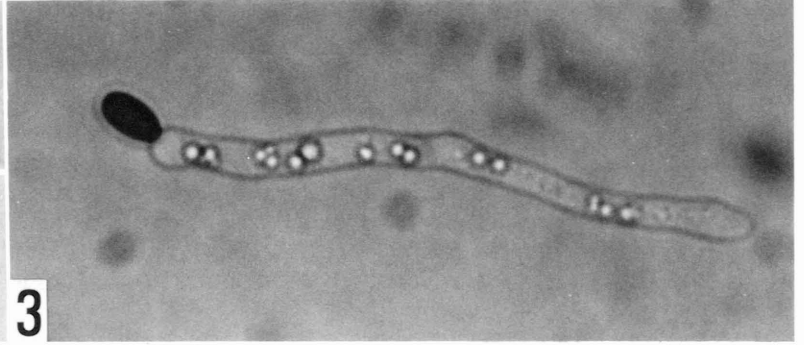
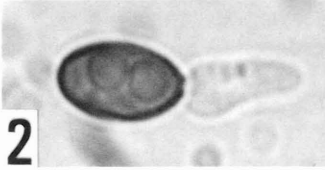
Little is known about sexuality in *Camarops* (Sphaeriales; Boliniaceae) due in part to the rarity of many species (10) and the inability to produce stromata in culture. The isolation of *Camarops petersii* (Berk. & Curt.) Nannfeldt [= *Peridoxylon petersii* (Berk. & Curt.) Shear] from germinating ascospores obtained from a stroma collected in Illinois led to attempts to produce stromata in culture. This paper deals with the successful production of fertile stromata of *C. petersii* on an agar medium.

Several stromata were collected from a decaying oak (*Quercus* sp.) log at Starved Rock State Park, near Utica, La Salle County, Illinois, 30-X-1982. The distinctive stromatal peridium, characteristic of *C. petersii* (5, 11), had sloughed away following rupture, leaving only a ring around the periphery of the stromata where it had originally been attached. *Camarops petersii* has not been reported previously from Illinois, although it has been collected in other areas of the eastern half of the U.S. (2, 5, 6, 8, 11). A portion of the collection was deposited with ILLS. A section one cm<sup>2</sup> was removed from the center of one of the stromata, crushed in sterile distilled water with a glass rod to remove ascospores, and filtered through glass wool. The suspension of ascospores was then diluted to  $5 \times 10^5$  spores/ml, spread (0.1 ml/plate) on malt extract agar (MEA; 3) (malt extract, 20 g; peptone, 1 g; dextrose, 20 g; agar, 25 g; distilled water, 1 liter) containing streptomycin (25 mg/l) and tetracycline (1.25 mg/l), and incubated at 25 C. Ascospore germination of approximately 5% occurred after 40-60 h. Germ tubes invariably originated from the minute germ pore present at the slightly acuminate end of the spore (FIGS. 1-3). Young colonies were transferred to slants of MEA and later stored at 5 C.

<sup>1</sup> The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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FIGS. 1-8. *Camarops petersii*. 1, 2. Germinating ascospores on MEA with germ tubes emerging from germ pores,  $\times 2500$ . 3. Young germling,  $\times 1300$ . 4. Colony on MEA (21 da, 25 C),  $\times 0.3$ . 5. Stroma from culture with peridium beginning to separate (exudate removed),  $\times 10$ . 6. Stroma with peridium completely folded back, exposing the ostioles of the perithecia (exudate removed),  $\times 10$ . 7, 8. Vertical section of stroma showing perithecia,  $\times 10$ ,  $\times 25$ , respectively.



A preliminary attempt to obtain stromata in culture was made by inoculating 100 × 15 mm plates of MEA, Sabouraud maltose agar (1), M4OY (Harrold's Agar; 7), potato dextrose agar with 0.5% yeast extract (4), and potato sucrose agar (20 g sucrose/l) with an isolate of *C. petersii*. Cultures were incubated in sealed plastic bags at 25 C in darkness for 16 wk. Although vegetative growth occurred on all media, stromata formed only on MEA. The reproducibility of these results was verified using three isolates from the original stroma (deposited in the Agricultural Research Culture Collection, Northern Regional Research Center, Peoria, Illinois, and designated as NRRL 13109, NRRL 13110, and NRRL 13111), each of which was inoculated separately onto 10 plates of MEA and incubated as above.

Growth of *C. petersii* was moderately rapid on MEA, with colonies attaining an average diam of 73 mm in 21 da (FIG. 4). Colonies were floccose, ranged from tan to deep brown, and produced a dark red-brown soluble pigment that diffused into the medium. In 7–9 wk, raised areas (immature stromata) covered with beads of light brown exudate appeared, particularly near the edge of the Petri dish. The peridia began to split apart in 9 wk, rupturing the overlying mycelium and peeling back to expose the ostioles of the stromatal perithecia (FIGS. 5, 6). Approximately 5 (0–10) stromata formed per plate, individual stromata averaging 5.4 (2–12.9) mm in diam. Dimensions of stromata were considerably smaller than the original stroma from which the isolates were obtained (35 × 40 mm). Stromata produced large amounts of exudate, which was dark brown due to the presence of ascospores. When stromata were sectioned, polystichous perithecia were observed with long, slender necks characteristic of the species (5, 8) (FIGS. 7, 8). Ascospore dimensions of (3–)3.3(–4) × (5.8–)6.8(–8) μm were similar to those from the original collection. The percentage of germination of ascospores was low (0.02%) when the spores were plated on MEA. Germination occurred at the apical germ pore, and germlings gave rise to typical *C. petersii* colonies.

Heagle and French (8) and Fergus (6) reported they were unable to germinate ascospores from stromata of *C. petersii* collected from natural substrates. The low germinability of ascospores from culture may indicate that the spores must undergo further maturation and/or require an environmental stimulus. The original stromata collected in this study appeared weathered and had undoubtedly been exposed to local environmental conditions, which may explain why some of the ascospores germinated.

Ascospores were not forcibly ejected from stromata produced in culture, but were extruded through the ostioles of the perithecia with a large amount of exudate. Nannfeldt (10) believed this exudate originates from early deliquescent paraphyses and asci, and that species of *Camarops* have lost the ability to discharge ascospores. However, Heagle and French (8) reported that ascospores of *C. petersii* are ejected at least 1 cm. The method of spore discharge might be affected by conditions under which stromata develop. Such is the case in *Hypoxylon serpens* (Pers.:Fr.) Kickx, where ascospores originating from stromata in culture either are extruded from the perithecium with ascus debris or are forcibly discharged, depending upon the composition of the growth medium (9).

*Camarops* is characterized by Nannfeldt (10) as lacking an anamorphic state. It is noteworthy that in culture *C. petersii* did not produce conidia on either the vegetative mycelium or the surface of stromata.

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Key Words: *Camarops petersii*, stroma, ascospore germination, *Peridoxylon*.

## LITERATURE CITED

1. **Anonymous.** 1953. *Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures*. 9th ed. Difco Laboratories, Detroit, Michigan. 350 p.
2. **Berkeley, M. J., and M. A. Curtis.** 1869. Fungi cubenses (Hymenomycetes). *J. Linn. Soc. Bot.* **10**: 280-392.
3. **Blakeslee, A. F.** 1915. Lindner's roll tube method of separating cultures. *Phytopathology* **5**: 68-69.
4. **Chacko, R. J., and J. D. Rogers.** 1981. Cultural characteristics of some species of *Xylaria*. *Mycologia* **73**: 415-428.
5. **Ellis, J. B., and B. M. Everhart.** 1888. Synopsis of the North American species of *Hypoxylon* and *Nummularia*. *J. Mycol.* **4**: 38-44.
6. **Fergus, C. L.** 1973. *Peridoxylon petersii* on oak in Pennsylvania. *Mycologia* **65**: 1199-1200.
7. **Harrold, C. E.** 1950. Studies in the genus *Eremascus*. I. The rediscovery of *Eremascus albus* Eidam, and some new observations concerning its life history and cytology. *Ann. Bot. (London)* **14**: 127-148.
8. **Heagle, A. S., and D. W. French.** 1972. *Peridoxylon petersii* on oak in Minnesota. *Mycologia* **64**: 1349-1351.
9. **Kenerley, C. M., and J. D. Rogers.** 1976. On *Hypoxylon serpens* in culture. *Mycologia* **68**: 688-691.
10. **Nannfeldt, J. A.** 1972. *Camarops* Karst. (Sphaeriales-Boliniaceae). *Svensk Bot. Tidskr.* **66**: 335-376.
11. **Shear, C. L.** 1923. Life histories and undescribed genera and species of fungi. *Mycologia* **15**: 120-131.